

Abstract No. grif304

Crystal Structure of a Protein Repair Methyltransferase from *Pyrococcus furiosus* with its L-Isoaspartyl Peptide Substrate

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Beamline(s): X8C

Introduction: Protein L-isoaspartyl (D-aspartyl) methyltransferases (E.C. 2.1.1.77) are found in almost all organisms. These enzymes catalyze the S-adenosylmethionine (AdoMet)-dependent methylation of isomerized and racemized aspartyl residues in age-damaged proteins as part of an essential protein repair process. We have reported crystal structures of the repair methyltransferase at resolutions of up to 1.2 Å from the hyperthermophilic archaeon *Pyrococcus furiosus* as Protein Data Bank entries 1jg1, 1jg2, 1jg3 and 1jg4.

Methods and Materials: Native crystals of the *P. furiosus* methyltransferase grew in 2-7 days at 24° C by the hanging drop method. One microliter of 12 mg/mL protein in a 0.5 M NaCl and 20 mM HEPES pH 7.0 buffer was mixed with one μ L of a well solution containing 25% polyethylene glycol 2000, 15% glycerol, 0.2 M sodium acetate pH 4.6. The mixture was then allowed to equilibrate over the well solution. The crystals belong to space group P2₁2₁2₁ (a = 39.2 Å, b = 52.5 Å, c = 96.6 Å). Candidates for heavy atom derivatives were screened by the gel shift method of Boggon and Shapiro.¹ Protein was incubated for 10 min at room temperature in 28 different one mM solutions of heavy metals and analyzed by native PAGE. A clear gel shift was found for samarium chloride. Native methyltransferase crystals were briefly soaked in samarium tetrachloride producing a derivative suitable for multiwavelength anomalous dispersion (MAD) phase determination.

Diffraction data (for structures with the PDB codes 1jg1, 1jg2 and 1jg4) were collected at Brookhaven National Laboratories using beamline X8C of the National Synchrotron Light Source and reduced using DENZO/SCALEPACK.² A single samarium derivative crystal was used to collect MAD data sets at three wavelengths, with the 1.819 Å inflection wavelength treated as a reference using the program SOLVE.³ Three samarium sites were found in the anomalous difference Patterson maps using SHELXD.⁴ After phasing with MLPHARE⁵ and applying density modification with DM^{5,6} a figure of merit of 0.76 was achieved and an interpretable density map at 2.1 Å was calculated with CNS.¹¹ The majority of the protein sequence was built into this map, after which automated model building was employed. The high quality of the native data set combined with the MAD phases permitted the program ARP (Automatic Refinement Program) to trace nearly the entire protein chain.⁷ A few rounds of refinement with SHELXL⁸ and the addition of 256 well-ordered water molecules led to a final structure with a working R-value of 14.7% and a free R value of 20%. The final structure was validated with Ramachandran plots and the programs ProCheck⁹ and Errat¹⁰. The AdoHcy structure was used as a starting model for refinement of the Adomet and adenosine structures using CNS.¹¹ Refinement continued with CNS¹¹ for several rounds with model adjustments in O.¹²

Results: Refined structures include binary complexes with the active cofactor AdoMet, its reaction product S-adenosylhomocysteine (AdoHcy), and adenosine. The enzyme places the methyl-donating cofactor in a deep, electrostatically negative pocket that is shielded from solvent. Across the multiple crystal structures visualized, the presence or absence of the methyl group on the cofactor correlates with a significant conformational change in the enzyme in a loop bordering the active site, suggesting a role for motion in catalysis or cofactor exchange. We also report the structure of a ternary complex of the enzyme with adenosine and the methyl-accepting polypeptide substrate VYP(L-isoAsp)HA at 2.1 Å.

Conclusions: The substrate binds in a narrow active site cleft with three of its residues in an extended conformation, suggesting that damaged proteins may be locally denatured during the repair process in cells. Manual and computer-based docking studies on different isomers help explain how the enzyme uses steric effects to make the critical distinction between normal L-aspartyl and age-damaged L-isoaspartyl and D-aspartyl residues.

Acknowledgments: This abstract is taken from a version of Griffith, S., Sawaya, M., Boutz, D., Thapar, N., Katz, J., Clarke, S. and Yeates, T.O. submitted to Journal of Molecular Biology in July 2001. The authors would like to thank Dr. Duilio Cascio and Cameron Mura for useful discussions. We also thank the members of the PRT for Brookhaven National Lab beamline X8C. This work was supported by USPHS Predoctoral Training Program GMO7185 to SCG and JEK, Department of Energy Grant DE-FC03-87ER60615 to TOY, and NIH grants AG18000 and GM26020 to SC.

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